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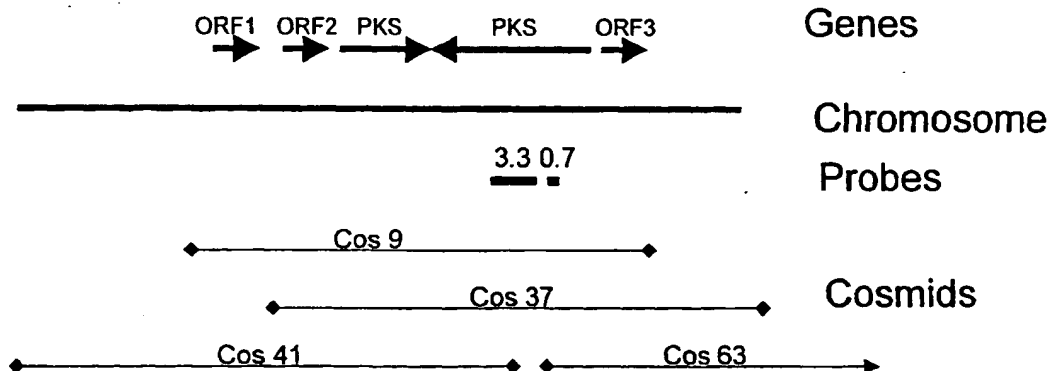
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(54) Title: GENES ENCODING ENZYMES IN THE BIOSYNTHESIS OF PIMARICIN AND THE APPLICATION THEREOF



(57) Abstract: A polynucleotide comprises the nucleic acid sequence set out in SEQ ID NO: 5, 7 or 9 or a homologue or fragment thereof or a sequence complementary thereto. Polynucleotides of the invention may be used for modifying the biosynthesis of pimaricin and also in the biosynthesis of new compounds.

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/00/06227

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/53 C12N15/52 C12N9/02 C12N9/04 C12P19/62

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, STRAND, EMBL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>APARICIO ET AL.: "The biosynthetic gene cluster for the 26-membered ring polyene macrolide pimarin. A new polyketide synthase organization encoded by two subclusters separated by functionalization genes"</p> <p>JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 15, 9 April 1999 (1999-04-09), pages 10133-10139, XP002120719 page 10134, column 2; figure 2 page 10137, column 2</p> <p style="text-align: center;">--- -/--</p>	1-13, 15-27

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 95 01098 A (MONSANTO CO) 12 January 1995 (1995-01-12)</p> <p>page 15, line 25 -page 16, line 29 page 16, line 15 - line 29 page 19, line 23 -page 24, line 31 seq id no 8 page 30 -page 32 table 3</p>	1-6,12, 13,15, 17,20, 21,23-27
X	<p>US 5 672 497 A (COX KAREN L ET AL) 30 September 1997 (1997-09-30)</p> <p>column 9, line 35 - line 64; figure 1; table 11.</p>	1-7,9, 10,12, 13, 15-17, 19-21, 23-27
A	<p>WO 98 11230 A (SQUIBB BRISTOL MYERS CO) 19 March 1998 (1998-03-19) seq id no 22 page 25, line 9 -page 27, line 30; claims 1-27; table 1</p>	1-27
P,X	<p>BRAUTASET ET AL: "Biosynthesis of the polyene antifungal antibiotic nystatin in Streptomyces noursei ATCC 11455: analysis of the gene cluster and deduction of the biosynthetic pathway" CHEMISTRY AND BIOLOGY, vol. 7, no. 6, 23 May 2000 (2000-05-23), pages 395-403, XP000953274 nysl page 401 -page 402; figure 4; table 2 &amp; DATABASE EMBL Online! EBI; ACC.NO.:AF263912, 25 May 2000 (2000-05-25) BRAUTASET ET AL.: "Streptomyces noursei ATCC 11455 nystatin biosynthetic gene cluster, complete sequence" XP002151299 nysl abstract</p>	1-6,12, 13,24-27

# INTERNATIONAL SEARCH REPORT

on patent family members

Interr. 1st Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9501098 A	12-01-1995	US 5518908 A AT 147231 T AU 686200 B AU 7214094 A BR 9406965 A CA 2163120 A CN 1126423 A DE 69401436 D DE 69401436 T DK 706320 T EP 0706320 A ES 2097656 T GR 3023050 T HU 73324 A JP 9500528 T NZ 268794 A PL 312277 A US 5554369 A US 5558862 A US 5763245 A	21-05-1996 15-01-1997 05-02-1998 24-01-1995 27-08-1996 12-01-1995 10-07-1996 20-02-1997 26-06-1997 07-07-1997 17-04-1996 01-04-1997 30-07-1997 29-07-1996 21-01-1997 27-08-1996 15-04-1996 10-09-1996 24-09-1996 09-06-1998
US 5672497 A	30-09-1997	AT 116364 T AU 603088 B AU 7040587 A BG 60053 A CN 87102137 A DE 3750907 D DE 3750907 T DK 135787 A EP 0238323 A ES 2067443 T GR 3015151 T HU 43631 A,B IE 65709 B IL 81892 A JP 2674998 B JP 62275686 A KR 9206350 B NZ 219694 A NZ 232685 A PT 84489 A,B RU 2043416 C ZA 8701862 A	15-01-1995 08-11-1990 24-09-1987 16-08-1993 04-11-1987 09-02-1995 18-05-1995 22-09-1987 23-09-1987 01-04-1995 31-05-1995 30-11-1987 15-11-1995 31-10-1995 12-11-1997 30-11-1987 03-08-1992 29-01-1992 29-01-1992 01-04-1987 10-09-1995 26-10-1988
WO 9811230 A	19-03-1998	NONE	